

Wip1 regulates the immunomodulatory effects of murine mesenchymal stem cells in type 1 diabetes mellitus *via* targeting IFN- α /BST2

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Abstract

Background: Mesenchymal stem cells (MSCs) shows significant therapeutic effects in type 1 diabetes mellitus (T1DM) as they could regulate the inflammatory processes. However, little is known about the process of MSCs immunosuppression in T1DM. In this study, we investigated the effects of wild type p53-induce phosphatase 1 (Wip1) on regulating MSCs immunosuppressive capacities in T1DM mice.

Methods: Primary wild type (*Wip1^{+/+}*) MSCs and Wip1 knockout (*Wip1^{-/-}*) MSCs were cultured *in vitro*. T1DM mouse model was induced with streptozotocin and then was treated with *Wip1^{+/+}* MSCs (5×10^5) or *Wip1^{-/-}* MSCs (5×10^5) by tail vein injection. The general physiological states of T1DM mice were measured every week. Moreover, the pathological changes in the pancreatic tissue were observed. Enzyme-linked immunosorbent assay (ELISA) and flow cytometry were used to detect the expressions of inflammatory cytokines in mice.

Results: *Wip1^{-/-}* MSCs had lower therapeutic effects in T1DM mice. Moreover, we screened and confirmed bone marrow stromal cell antigen2 (*BST2*) gene that showed the target gene for *Wip1* through gene chips, quantitative polymerase chain reaction and Western blot. *Wip1^{-/-}* MSCs exhibited lower immunosuppressive capacity, as evidenced by enhanced expression of *BST2*, with concurrent increased expression of interferon- α (IFN- α). *In vivo* distribution analysis results indicated that *Wip1^{-/-}* MSCs homed to the damaged pancreatic tissue. *Wip1^{-/-}* MSCs influenced the expression of immune factors by remarkably increasing the expression of tumor necrosis factor- α (TNF- α), interleukin-17A (IL-17A), IFN- α , IFN- β , and IFN- γ and decreasing the expression of IL-4 and IL-10.

Conclusions: *Wip1* affects MSCs immunomodulation by regulating the expression of IFN- α /*BST2*. These findings suggest that *Wip1* is required to regulate the therapeutic effects of MSCs on T1DM treatment, indicating a novel role of *Wip1* in immunoregulation.

Background

Type 1 diabetes mellitus (T1DM), an autoimmune disease induced by multiple factors, causes pancreatic infiltration of T lymphocytes and destruction of β islet cells, which ultimately leads to a significant decline in insulin release[1–3]. Currently, mesenchymal stem cells (MSCs) have been extensively utilized in treating T1DM and its complications as they contribute to the alleviation of autoimmune response and apoptotic delay in β islet cells[4, 5]. However, little is known about the therapeutic effects of MSCs on T1DM.

Wild type p53-induce phosphatase 1 (Wip1), encoded by the *PPM1D* gene, is a serine/threonine phosphatase newly identified in wild-type p53-induced phosphatase of the PP2C family[6]. It has been reported to be closely associated with tumorigenesis, cell proliferation, as well as development and aging processes[7]. Meanwhile, it played important roles in regulating the function of immune cells[8, 9]. Recently, several studies demonstrated that Wip1 could modulate MSCs migration, proliferation, and

senescent growth arrest[10, 11]. In addition, *Wip1* knockdown in mouse embryonic fibroblasts led to reduction of insulin-regulated AKT activity in diabetes[12]. Furthermore, *Wip1* regulated islet cell proliferation and regeneration through modulating the p38 MAPK pathway[13]. These indicated that *Wip1* was essential for the biological characteristics of MSCs and islet cells. Nevertheless, the effects of *Wip1* on the immunomodulatory function of MSCs remain unclear.

To investigate the effects of *Wip1* on immunosuppressive properties of MSCs and the therapeutic effects of T1DM, we isolated MSCs from *Wip1*^{-/-} mice. These MSCs showed weak anti-inflammatory activity in T1DM mice through increasing the expression of bone marrow stromal cell antigen2 (*BST2*). In addition, *BST2* contributed to the high expression of interferon- α (IFN- α) in *Wip1*^{-/-} MSCs, which was an important factor in the pathogenesis of T1DM. Importantly, administration of *Wip1*^{-/-} MSCs to T1DM mice potently promoted IFN- α expression and aggravation of inflammatory responses in the pancreatic microenvironment. On this basis, *Wip1* played vital roles in the therapeutic effects of MSCs in T1DM mice, which provided a novel mechanism for understanding the immunosuppressive capacity of MSCs.

Materials And Methods

Mice

C57BL/6j mice (6–8 weeks, 20 g) were purchased from Vital River Laboratory Animal Technology (Beijing, China). *Wip1*^{-/-} mice were raised in specific pathogen free (SPF) room in the Advanced Laboratory Animal Center, the Academy of Military Medical Sciences (Beijing, China). All animals were fed with sterilized food and drinking water, and were handled according to the Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Committee on the Ethics of Animal Experiments, the Academy of Military Medical Sciences.

T1DM induction and experimental therapies

To induce T1DM model, male C57BL/6j mice were fed ad libitum for 48 h followed by food and water deprivation for 10–16 h before T1DM induction. Subsequently, streptomycin (STZ, 50 mg/kg) dissolved in sodium citrate buffer was administered by intraperitoneal injection in the lower left abdomen once per day for 5 days. The ad libitum diet was resumed immediately after the first injection. T1DM was defined as a blood glucose of ≥ 16.7 mmol/L for three consecutive random samplings.

After T1DM induction, *Wip1*^{+/+} MSCs (5×10^5), *Wip1*^{-/-} MSCs (5×10^5) and PBS (0.2 ml) were intravenously administered to T1DM mice on day 1 and 14, respectively. After treatment, the morphology, activity, fur, and food and water intake were monitored for each mouse. Random blood glucose and body weight were measured per week. For tissue and immune cell analysis, mice were euthanized on day 28.

Cells preparation

Murine primary MSCs were isolated from mouse compact bone and identified according to the previous description[14]. One-week-old mouse pups were collected and tailed for genotyping. The femur and tibia

of 1-week-old *Wip1^{+/+}* and *Wip1^{-/-}* mice were isolated under aseptic conditions. The bone fragments were digested using type II collagenase at 37 °C for 45 min. Afterwards, the mixture was transferred to α -MEM complete medium containing 10% fetal bovine serum (FBS). Adherent cells were maintained with medium replenishment every 3 days. The 3rd passage cells were used for measuring differentiation characterization.

Flow cytometry

Flow cytometry was used for the analysis of cell surface markers. Cells were resuspended using PBS, and then were labeled with antibodies including FITC anti-mouse MHCII, FITC anti-mouse CD11b, FITC anti-mouse Sca-1, FITC anti-mouse CD34, FITC anti-mouse CD45, PE anti-mouse CD31, PE anti-mouse CD29, or PE anti-mouse CD90. Subsequently, the cells were incubated in dark for 30 min, followed by washing with PBS. Next, FACS Calibur (BD Biosciences) was used to measure cell fluorescence. Flow cytometry data were analyzed using FlowJo.

For intracellular staining, splenic lymphocytes were collected from mice and stimulated for 4–5 h with Cell Stimulation Cocktail (eBioscience, catalog No.:00-4970-03c), then the cells were stained with FITC-CD3 (eBioscience, catalog No.:11-0032-80) and PE-CD4 (eBioscience, catalog No.:MCD0404) for 30 min at 4 °C. Cells were then fixed, permeabilized and stained for transcription factors using the Fixation/Permeabilization Diluent (eBioscience, catalog No.:00-5223-56) according to the manufacturer's instructions. For cytokine staining, the cells were stained with APC-IFN- γ and measured with flow cytometry. Data were analyzed with FlowJo software.

MSCs staining

Wip1^{+/+} MSCs and *Wip1^{-/-}* MSCs were harvested after centrifugation, followed by aspirating the supernatant. Then the cells were resuspended gently in CellTracker™ CM-Dil (1:1000, Invitrogen) staining solution, followed by incubating for 30 min at 37 °C. The stained cells resuspended with PBS were injected to T1DM mice.

Histology analysis

Pancreatic sections were prepared from at least five mice in each group for hematoxylin-eosin (HE) staining. Mouse insulin antibody (1:100, clone C27C9; catalog No. 3014; Cell Signaling Technology) was used for immunohistochemical and immunofluorescent staining of sections.

Cell transfection

EGFP-*Wip1* and EGFP plasmids (1 μ g) were transiently transfected into 293T cells using jetPRIME for 48 h. Transfection efficiency was measured by flow cytometry. *Wip1^{-/-}* MSCs (P2, 1×10^5 /well) were seeded into six-well plates. Then BST2 siRNA and negative control (50 nM, Sangon Biotech) were transfected into the cells using jetPRIME for 48 h.

Gene knockdown using siRNA

BST2 expression was knocked down in *Wip1*^{-/-} MSCs using *BST2*-targeting siRNA. The siRNA target sequences for *BST2* were: 5'-AGG CCG AGA CAC AGG CAA ATT-3'; 5'-AGG AGU CCC UGG AGA AGA ATT-3'; 5'-GAG AAU CUG AGG AUC CAA ATT-3'. The siRNA target sequences were transfected into *Wip1*^{-/-} MSCs using jetPRIME for 48 h.

Cytokine measurement

Levels of tumor necrosis factor- α (TNF- α), interleukin-17 α (IL-17 α), IL-4, and IL-10 in pancreas supernatant and IFN- γ , IL-4, and IL-17A in the serum of T1DM mice treated with *Wip1*^{+/+} MSCs or *Wip1*^{-/-} MSCs were determined by ELISA (Invitrogen, CA, USA). The level of IFN- α in *Wip1*^{+/+} MSCs and *Wip1*^{-/-} MSCs culture supernatant was measured by ELISA kits, according to the manufacturer's instructions.

Western blotting

Total protein was extracted from the cell pellet with RIPA lysis buffer (eBioscience, catalog No.: 89901). The protein concentration was determined by the BCA method using commercial kit (Bio-Rad, catalog No.: M60-009RDPD). Protein lysis was separated by SDS-PAGE gel, and then was transferred to a PVDF membrane. The PVDF membrane was blocked for 2 h in TBST buffer containing 5% skim milk powder and then tested with *Wip1* Rabbit mAb (CST, catalog No.: 11901), *BST2* Rabbit mAb (CST, catalog No.: 19277) and β -Actin Rabbit mAb (CST, catalog No.: 4970). Proteins were visualized using HRP-conjugated secondary antibodies. The bands were then exposed by ECL software (Thermo Scientific).

Quantitative polymerase chain reaction (qPCR)

Total RNA was extracted from cultured cells using the TRIzol Reagent (Invitrogen, catalog No.: 15596018) and reverse-transcribed using SuperRT cDNA Synthesis Kit (CW BIO, catalog No.: CW0741). qPCR amplification was performed on 7500 Real-Time system. The qPCR primers purchased from Sangon (Sangon Biotech) were listed in **Table S1**. Relative quantification was performed using mouse-specific GAPDH primers.

Statistical analysis

Data were presented as mean \pm standard error of mean (SEM). The statistical significance of the differences between treatment groups was analyzed by Student's t-test or one-way analysis of variance. Data analysis was performed using GraphPad Prism Version 6.0 software. $P < 0.05$ was considered to be statistically significant.

Results

Isolation and identification of *Wip1*^{-/-} MSCs from murine compact bones

To investigate the roles of *Wip1* in the immunoregulatory function of MSCs for treating T1DM, MSCs were isolated from *Wip1*^{+/+} and *Wip1*^{-/-} mice by cultivating the digested compact bone. Fibroblast-like

cells sprouted from the bone fragments and adhered to the flask 48 h (Fig. 1A, Fig. S1). These adherent cells could be readily expanded *in vitro* by successive cycles of trypsinization, seeding, and culture every 3 days without visible morphologic alteration (Fig. 1B and 1C).

We then determined the adipogenic and osteogenic differentiation of *Wip1*^{-/-} cells *in vitro*. As shown in Fig. 1D and 1E, many Oil Red-O-positive lipid droplets were available in murine *Wip1*^{-/-} MSCs. To further determine the effects of *Wip1* on MSCs adipogenic differentiation, the mRNA levels of adipogenic markers, including C/EBP α and peroxisome proliferator-activated receptor γ (PPAR γ) were examined by qPCR. Consistent with histochemical staining results, the mRNA expression of *C/EBP α* and *PPAR γ* in *Wip1*^{-/-} MSCs showed elevation (Fig. 1F). Under osteogenic conditions for 2 weeks, the adherent cells from *Wip1*^{-/-} mice displayed alkaline phosphatase activity (Fig. 1G and 1H). In line with the results of differentiation assays, qPCR demonstrated transcriptional expression of Osteocalcin and Runx2 under specific osteogenic cultures in adherent cells (Fig. 1I).

The immunophenotype of the *Wip1*^{-/-} adherent cells was assessed by flow cytometry. The culture-expanded adherent cells were positive for CD90, CD29, and Scal-1 but were negative for CD31, MHCII, CD11b, CD34, and CD45 (Fig. 1J).

The morphologic, immunophenotypic, and differentiation assays strongly indicated that the adherent cells isolated from *Wip1*^{-/-} mice were MSCs. In addition, *Wip1*^{+/+} MSCs showed similar biological characteristics (data not shown).

Therapeutic effect of *Wip1*^{-/-} MSCs in T1DM mice

To assess the therapeutic effect of *Wip1*^{-/-} MSCs in T1DM mice, *Wip1*^{+/+} MSCs (5×10^5) and *Wip1*^{-/-} MSCs (5×10^5) were intravenously administered to STZ-induced T1DM mice on day 1 and 14. The general conditions of mice including body size, body weight, and blood glucose level were monitored. As shown in Fig. 2A, the body size in *Wip1*^{-/-} MSCs mice was smaller compared with *Wip1*^{+/+} MSCs mice. In T1DM mice that received MSCs, the blood glucose levels were lower in *Wip1*^{-/-} MSCs group compared with those of *Wip1*^{+/+} MSCs group (Fig. 2B). Additionally, the slow gain in weight caused by hyperglycemia was controlled by MSCs infusion. The average body weight of the *Wip1*^{+/+} MSCs group was larger than that of the *Wip1*^{-/-} MSCs group (Fig. 2C).

To further assess whether *Wip1*^{-/-} MSCs infusion could reduce the pathological damages in T1DM mice, the histological examination of islet tissues was performed by HE staining and immunohistochemical staining on day 28. As shown in Fig. 2D and 2E, islets of *Wip1*^{-/-} MSCs group exhibited mild inflammation. Additionally, there were a few of islet areas with preserved islet morphology, indicating that *Wip1*^{-/-} MSCs infusion could prevent the destruction of islets in T1DM. Moreover, the islet area and the mean density of insulin-positive cells in pancreatic tissues treated with *Wip1*^{+/+} MSCs were larger than

that of *Wip1*^{-/-} MSCs group, despite that the effects of *Wip1*^{-/-} MSCs were better than T1DM mice (Fig. 2F and 2G). These findings indicated the therapeutic effects of *Wip1*^{-/-} MSCs were weaker than *Wip1*^{+/+} MSCs in T1DM mice.

Wip1^{-/-} MSCs failed to reduce the inflammatory response in T1DM mice

Autoimmune diabetes is characterized by a Th1 immune response. Previous studies indicated that the immunoregulation of MSCs was closely involved in the inflammatory processes in T1DM[15, 16]. To investigate whether *Wip1* gene regulated the immunomodulatory function of MSCs in T1DM, flow cytometry was used to detect the intracellular inflammatory factor IFN- γ in Th1 cells (CD4⁺INF- γ ⁺ T cells) of spleen lymphocytes in each group. As shown in Fig. 3A and 3B, the percentage of Th1 cells (CD4⁺INF- γ ⁺ T cells) in T1DM group was significantly higher than that in other groups ($P < 0.01$). *Wip1*^{+/+} MSCs transfusion significantly decreased the percentage of Th1 cells ($P < 0.05$). After treating with *Wip1*^{-/-} MSCs, the percentage of Th1 (CD4⁺INF- γ ⁺ T cells) cells was lower than that of T1DM group, and was higher than that of *Wip1*^{+/+} MSCs group ($P < 0.05$).

Subsequently, we investigated whether *Wip1* could affect the expression of lymphocyte-derived immune factors mediated by MSCs infusion. Compared with *Wip1*^{+/+} MSCs group, serum IFN- γ and IL-17A showed significant increase and the IL-4 expression showed significant decline after *Wip1*^{-/-} MSCs infusion (Fig. 3C). These data indicated that *Wip1* gene may involve in the immunomodulatory activity of MSCs in T1DM mice.

Wip1 interacted with BST2

To investigate the involvement of *Wip1* in MSCs immunomodulatory activity, we then determined the gene expression profiles in *Wip1*^{+/+} MSCs and *Wip1*^{-/-} MSCs by microarray analysis. Bioinformatics methods were used to analyze the microarray results, which predicted that 23 genes were associated with immunomodulatory responses. Among the up-regulated genes in *Wip1*^{-/-} MSCs, *BST2* was identified to be related to inflammatory processes, and highly expressed in *Wip1*^{-/-} MSCs by qPCR assay (Fig. 4A and B). To further explore whether *BST2* was up-regulated in *Wip1*^{-/-} MSCs, we examined the expression of *Wip1* in various genotype mice. As shown in Fig. 4C and 4D, the protein expression level of *Wip1* in *Wip1*^{-/-} MSCs was lower than that of *Wip1*^{+/+} MSCs. Then we analyzed the *BST2* protein expression in *Wip1*^{-/-} MSCs, which showed that the protein expression of *BST2* was significantly up-regulated in *Wip1*^{-/-} MSCs (Fig. 4E and 4F).

Subsequently, we further investigated whether *Wip1* had direct interaction with *BST2*. The 293T cells were transfected with *Wip1*-EGFP for 48 h, followed by determining the expression of *Wip1* and *BST2*. GFP expression indicated high transduction efficiency, which showed a transfection efficiency of $61 \pm 10.81\%$

and $69 \pm 7.93\%$ for the EGFP group and Wip1-EGFP group, respectively (Fig. 4G and 4H). As shown in Fig. 4I, 293T cells transduced with Wip1-EGFP exhibited higher Wip1 expression and lower BST2 expression. Here, our data revealed that there was interaction between Wip1 and BST2.

High expression of BST2 can significantly promote the expression of IFN- α in Wip1^{-/-} MSC

BST2 was reported to activate plasma cell-derived dendritic cells (pDC) and promote IFN- α expression[17]. As an important inflammatory response factor, IFN- α involved in the regulation of the biological characteristics of MSCs and promoted the inflammatory response of T1DM[18–20]. Therefore, we explored whether BST2 could affect the regulation of Wip1^{-/-} MSCs on the inflammatory process in T1DM mice *via* IFN- α . As shown in Fig. 5A, the mRNA expression of IFN- α in Wip1^{-/-} MSCs was significantly higher than that of Wip1^{+/+} MSCs. Compared with Wip1^{+/+} MSCs, the protein expression of IFN- α in Wip1^{-/-} MSCs showed a dramatic increase (Fig. 5B). To investigate whether IFN- α was dependent on the BST2 expression in Wip1^{-/-} MSCs, we transfected Wip1^{-/-} MSCs with BST2 siRNAs or negative control. The results showed that BST2 knockdown in Wip1^{-/-} MSCs could significantly down-regulate the expression of IFN- α ($P < 0.05$, Fig. 5C). This implied that knockdown of BST2 could effectively inhibit the expression of IFN- α . On this basis, we demonstrated that BST2 was a key gene for regulating IFN- α expression in Wip1^{-/-} MSCs.

IFN- α secreted by Wip1^{-/-} MSCs aggravated inflammatory response in the pancreatic microenvironment

Several studies had shown that MSCs preferentially homed to the sites of tissue damages, where they enhanced wound healing, and involved in modulating the balance of inflammatory response[21]. To determine whether Wip1^{-/-} MSCs could migrate to the damage sides in pancreas, red fluorescence stained Wip1^{-/-} MSCs and Wip1^{+/+} MSCs were administrated into T1DM mice. After staining for 72 h, red fluorescence was occurred around blood vessels in pancreatic tissues (Fig. 6A), which indicated that Wip1^{-/-} MSCs could home to the pancreatic tissues in T1DM mice.

IFN- α has been well acknowledged to regulate the innate immune response and promote the adaptive immune response. Thus, it played important roles in the regulation of immune disorders. To explore whether IFN- α was secreted by Wip1^{-/-} MSCs, the expression of IFN- α in the pancreatic supernatant of T1DM mice in each group was detected by ELISA. The expression of IFN- α in the pancreatic supernatant of Wip1^{-/-} MSCs group showed significant increase ($2,103.14 \pm 797.1$ pg/mL) compared with the control group and Wip1^{+/+} MSCs group ($P < 0.05$). Compared with the T1DM model group, there was no significant difference. These data demonstrated that Wip1^{-/-}MSCs could promote IFN- α secretion after homing to pancreatic tissue (Fig. 6B). Besides, we further examined the expression of IFN- α , IFN- β and

IFN- γ in splenic lymphocytes. The expression of IFN- α , IFN- β and IFN- γ in *Wip1*^{-/-} MSCs group was higher than that of *Wip1*^{+/+} MSCs group, which indicated that *Wip1*^{-/-} MSCs also could promote the expression of IFN- α , IFN- β and IFN- γ in spleen lymphocytes, and further aggravate the inflammatory response(Fig.S2).

The expression of inflammatory factors (e.g. TNF- α , IL-17A, IL-4 and IL-10) in the pancreas supernatant in T1DM mice was measure by ELSIA. The expression of TNF- α (1,027.87 \pm 82.9 pg/ml) and IL-17A (519.02 \pm 47.01 pg/ml) in T1DM group were significantly higher than those in other groups (P < 0.05). After treating with *Wip1*^{+/+} MSCs, the expression of TNF- α (827.03 \pm 102.08 pg/ml) and IL-17A (283.53 \pm 48.26 pg/ml) showed significant decline compared with those in T1DM group (P < 0.05), while the expression of TNF- α (995.24 \pm 562.61 pg/ml) and IL-17A (413.33 \pm 42.98 pg/ml) in *Wip1*^{-/-} MSCs treatment group was significantly higher than those in *Wip1*^{+/+} MSCs group (P < 0.05). However, their levels were still lower than those in T1DM group. The expression of IL-4 (50.25 \pm 28.14 pg/ml) and IL-10 (1575 \pm 579.38 pg/ml) in the pancreas of the *Wip1*^{+/+} MSCs group was significantly higher than those of other groups (P < 0.05). IL-4 (7.35 \pm 3.5 pg/ml) and IL-10 (447.5 \pm 160.17 pg/ml) expression in the *Wip1*^{-/-} MSCs group was significantly lower than those in the *Wip1*^{+/+} MSCs group (P < 0.05). These data indicated that IFN- α secreted by *Wip1*^{-/-} MSCs could aggravate the inflammatory response in the pancreatic microenvironment (Fig. 6C).

Discussion

T1DM is a public health challenge worldwide with a hallmark of autoimmune attack of β -cells leading to specific loss of the insulin-secreting cells[22]. Previous clinical studies demonstrated that moderate immunosuppression in T1DM could prevent further loss of insulin production, which then attenuated the clinical symptoms[23–25]. With the capacity to modulate immune responses, MSCs have been widely employed to treat various inflammatory diseases including T1DM[25–27], however, the potential mechanisms remain largely elusive.

The present study provided a report on alterations of the immunomodulatory functions of MSCs by *Wip1* in T1DM *via* targeting IFN- α /BST2. Also, we provided a new mechanistic insight in the *Wip1* regulated the immunomodulatory function of MSCs based on *in vitro* and *in vivo* evidence. *Wip1*^{-/-}MSCs had lower therapeutic effects in T1DM model, which was featured by failure in reducing the inflammatory response and up-regulation of BST2 and IFN- α production.

Wip1 is a serine/threonine phosphatase expressed in hematopoietic progenitors, stem cells, neutrophils, macrophages, B and T lymphocytes[28]. Recent studies demonstrated that *Wip1* could control immune response through regulating the growth and biological function of immune cells. Moreover, *Wip1* was crucial for the modulation of MSCs migration, but little is known about the effects of *Wip1* on the immunosuppression capacity of MSCs. In a current study, we showed that the therapeutic effect of *Wip1*^{-/-} MSCs on T1DM in mice was significantly lower than that of the *Wip1*^{+/+} MSCs group. *Wip1*^{-/-}

MSCs triggered no decline of the Th1 frequency, and the level of serum[29] and IL-17 was nearly the same in T1DM mice. Previously, Th1 proinflammatory autoimmune response led to a failure of immune tolerance to β -cells[25], while MSCs decreased the frequency of Th1 cells and reduced IFN- γ and IL-17 concentrations in T1DM. Therefore, the immunosuppressive effects of MSCs on T1DM were more likely to depend on *Wip1*. However, little is known about the molecular mechanism involving in the MSCs immunomodulation by *Wip1*. The combination of gene chips and bioinformatics method was utilized to analyze the target genes, which showed that *BST2* was the target gene for *Wip1*. Moreover, Western blot was used to detect the expression of *BST2* in *Wip1*^{+/+} MSCs and *Wip1*^{-/-} MSCs, which showed that *Wip1* could inhibit the expression of *BST2*. Previous studies reported that the expression of *BST2*, serving as a surface protein involved in viral vesicle budding in response to interferons[30]. Our data also indicated that the expression of IFN- α in *Wip1*^{-/-} MSCs was significantly up-regulated in protein and gene levels. *BST2* siRNA was further synthesized and transfected into *Wip1*^{-/-} MSCs. The results showed that *BST2* deficiency significantly inhibited the expression of IFN- α . These above results confirmed for the first time that *Wip1*^{-/-} MSCs could regulate the expression of IFN- α by regulating its endogenous *BST2* expression.

IFN- α , encoded by the type 1 interferon gene, is known to be crucial for the pathogenesis of autoimmunity[31, 32]. Clinical studies indicated that IFN-I, particularly IFN- α , and the *IFN* gene signature had been detected in islets and peripheral blood of T1DM patients, which contributed to the evolution of autoimmunity causing damages to β cells. However, little is known about the roles of IFN- α in the therapeutic effects of MSCs on T1DM. In this study, IFN- α expression was higher in *Wip1*^{-/-} MSCs. According to the previous studies, MSCs could preferentially home to damage sites in certain tissues, which then enhanced the wound healing and tissue regeneration, and inhibited the inflammatory reprocess[33, 34]. Our *in vivo* data showed that *Wip1*^{-/-} MSCs could home to pancreatic tissues in T1DM mice, leading to increase of IFN- α , IL-17a and TNF- α , and decrease of IL-4 and IL-10. This exacerbated the inflammatory response in the microenvironment of pancreatic and deterioration of clinical symptoms in T1DM mice. Therefore, our studies, together with previous investigations, demonstrated that there was high expression of IFN- α in *Wip1*^{-/-} MSCs, which was mediated by *BST2*. However, in our study, the exact mechanisms of how *Wip1* regulates *BST2* in MSCs are still not well defined, which requires further investigation.

In summary, *Wip1* contributed to IFN- α expression in MSCs via increasing the expression of *BST2*, which thereby impaired the therapeutic effects of MSCs in T1DM mice. *Wip1*^{-/-} MSCs migrated to the pancreas in T1DM mice, which led to secretion of IFN- α and augmentation of inflammatory processes. Additionally, we indicated the importance of *Wip1*-*BST2*-IFN- α axis in the therapeutic effects of MSCs in T1DM mice.

Conclusion

This study identified the role of the *Wip1* gene in regulating the immunomodulatory capabilities of MSCs. Furthermore, *Wip1* was shown to directly impact the immunosuppression effect of MSCs within T1DM mice. The mechanism of *Wip1* in MSCs regulation of immunological function is pivotal for claiming the

Wip1-BST2-IFN- α signal pathway in the therapeutic effects of MSCs in T1DM mice. These results may help in the development of cell-based therapies for the treatment of T1DM.

Declarations

Acknowledgements

Not applicable.

Author contributions

ZN and LWJ design the study, performed all research work, and analyzed the results and wrote this manuscript; LYL carried out the cell cultures and performed related assays; LX and WY performed the study; ZRX designed the study, and wrote the paper. Yi Zhang designed the study, analyzed data, prepared figures, wrote the paper, administrative support, and final approval of manuscript.

All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that they have no competing interests.

Availability of data and materials

All the data were available upon appropriate request.

Ethics approval and consent to participate

The study was conducted according to the Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Committee on the Ethics of Animal Experiments, the Academy of Military Medical Sciences.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Abbreviations

MSCs: mesenchymal stem cells; T1DM: type 1 diabetes mellitus; Wip1: Wild type p53-induce phosphatase 1; *Wip1^{+/+}* MSCs: wild type MSCs; *Wip1^{-/-}* MSCs: Wip1 knockout MSCs; BST2: bone marrow stromal cell antigen2; IFN- α : interferon- α .

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Figures

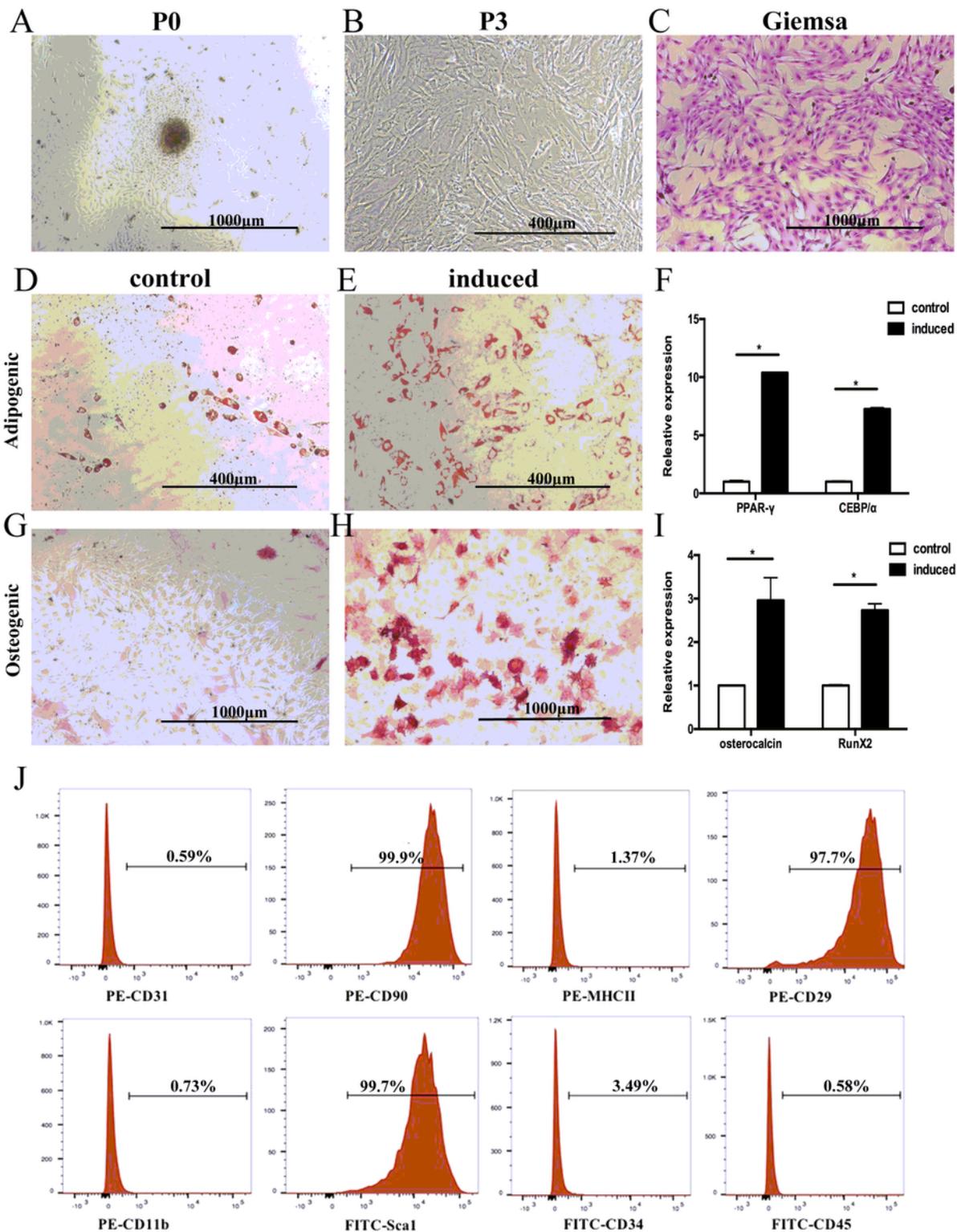


Figure 1

Isolation and identification of *Wip1*^{-/-} MSCs from murine compact bones. (A) Nucleated cells isolated from compact bones of *Wip1*^{-/-} mice became adherent 72 h after seeding. (B) The adherent cells (P3) displayed fibroblast-like morphology. The adherent cells were stained with Wright-Giemsa. (C) Multilineage differentiation potential of compact bones-derived adherent cells in *Wip1*^{-/-} mice was assessed by inducing adipogenic or osteogenic capacities in vitro. (D, E) Adipogenesis differentiation was

indicated by the presence of lipid drops stained with oil red O. (G, H) Osteogenic differentiation was shown by intracytoplasmic accumulation of alkaline phosphatase. (F, I) The expression of PPAR- γ and CEBP β in the adipogenic induction group and Osteocalcin and Runx2 in the osteogenic induction group was detected by qPCR (n = 3). (J) Immunophenotyping of culture-expanded adherent cells from Wip1-/- mice compact bones-derived adherent cells was analyzed by flow cytometry. *P < 0.05, compared with control group.

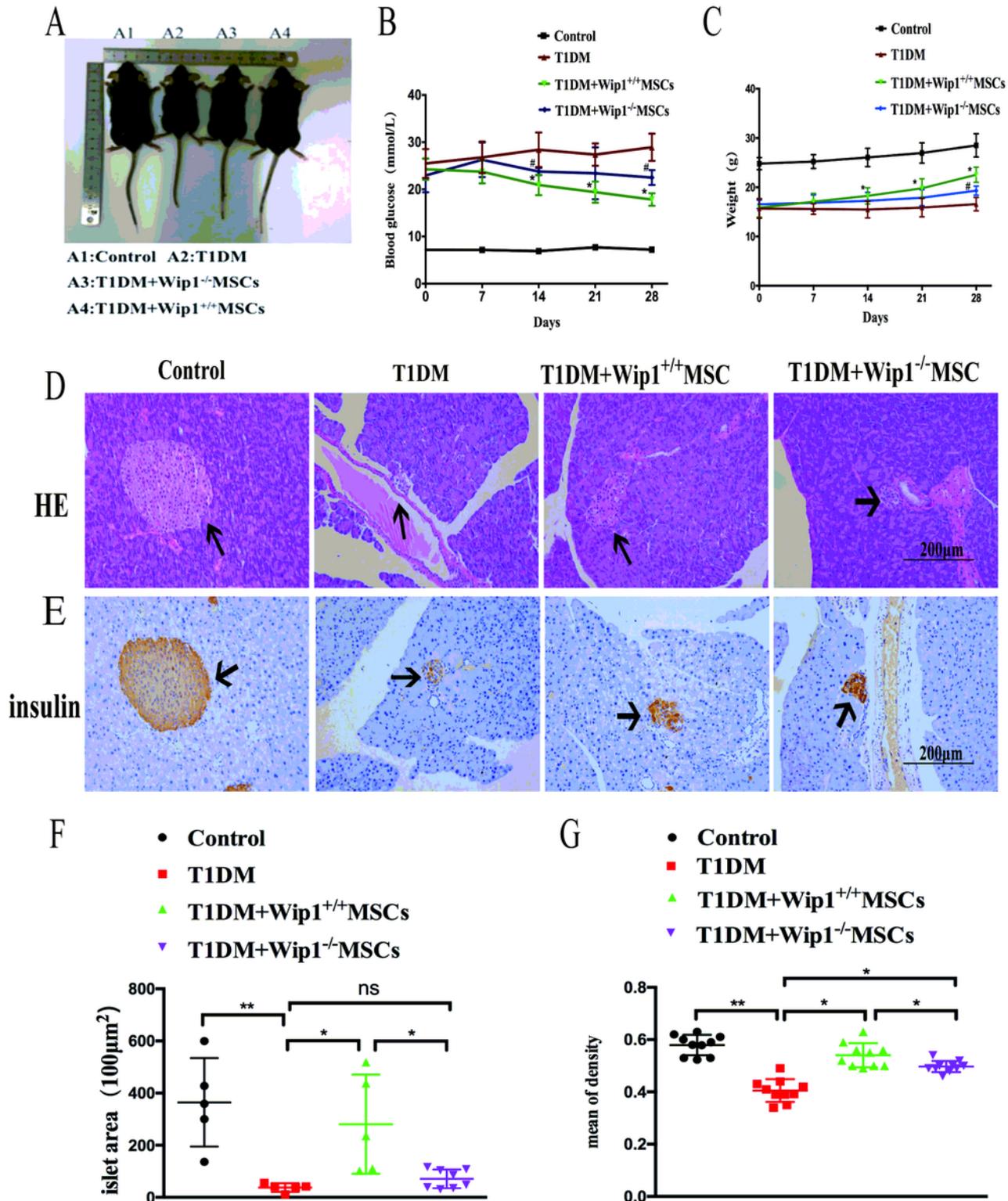


Figure 2

Wip1 knockout impaired the therapeutic efficacy of MSCs in T1DM mice. (A) The body size of mice after infusion of Wip1^{+/+} MSCs and Wip1^{-/-} MSCs. (B, C) The blood glucose and the weight curves (n=8). (D, E) Pathological damages in pancreatic tissue by H&E-staining and immunohistochemical staining. (F, G) The islet area and the mean density of insulin-positive cells in pancreatic tissues treated with Wip1^{-/-} MSCs were larger than that of Wip1^{+/+} MSCs group (n=8).

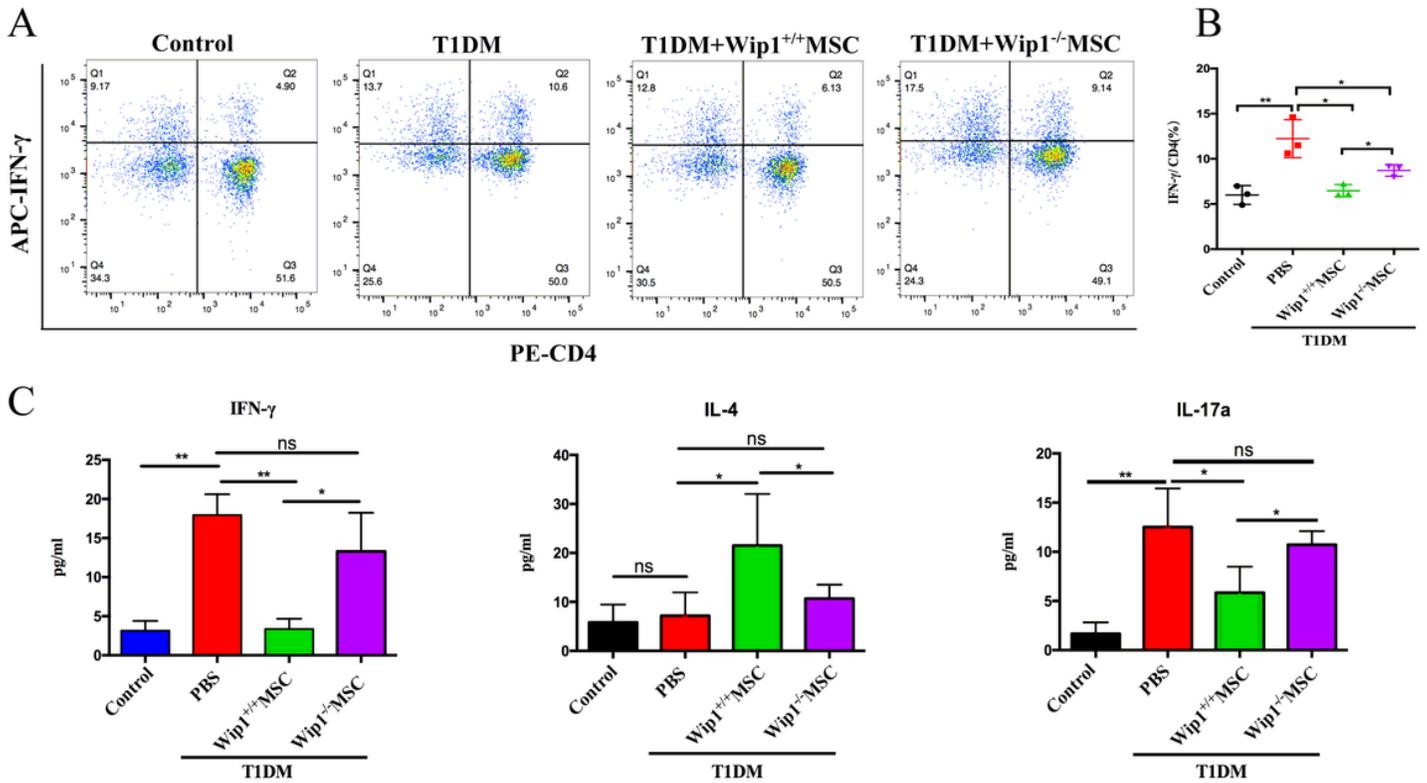


Figure 3

Wip1^{-/-} MSCs exhibited lower immunosuppression response in T1DM mice. (A) Frequency of CD4⁺IFN- γ ⁺ Th1 cells was analyzed by flow cytometry in MSCs-treated T1D mice. (B) The quantitative results for CD4⁺IFN- γ ⁺ Th1 cells in splenic lymphocytes. (C) Serum IFN- γ , IL-17a and IL-4 were detected by ELISA (n=7). *P < 0.05, **P < 0.01.

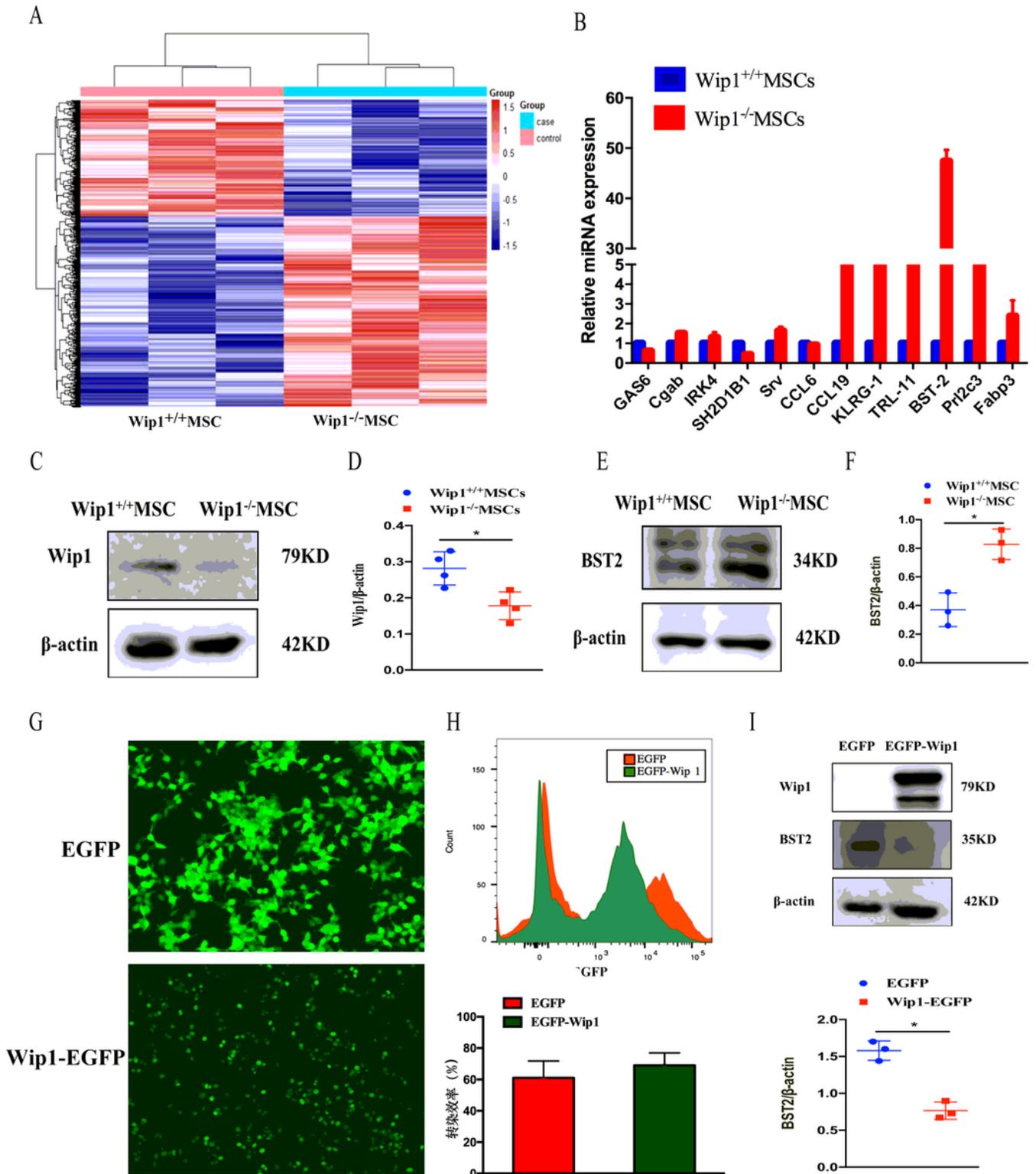


Figure 4

Wip1 interacted with BST2. (A) The RNA transcripts heatmap of Wip1^{+/+} MSCs and Wip1^{-/-} MSCs was displayed by Microarray analysis. (B) The upregulated genes in Wip1^{+/+} MSCs and Wip1^{-/-} MSCs were determined by qPCR. (C-F) Western blot was used to confirm the protein expression of Wip1 and BST2 in Wip1^{+/+} MSCs and Wip1^{-/-} MSCs. (G) Wip1-EGFP and EGFP plasmids were transfected into 293T cells, and the positive cells were observed by fluorescence microscope. (H) The transfection efficiency of Wip1-

EGFP and EGFP plasmids in 293T cells was detected by flow cytometry. (I) Western blot assay was utilized to determine the endogenous interaction between Wip1 and BST2 in 293T cells.

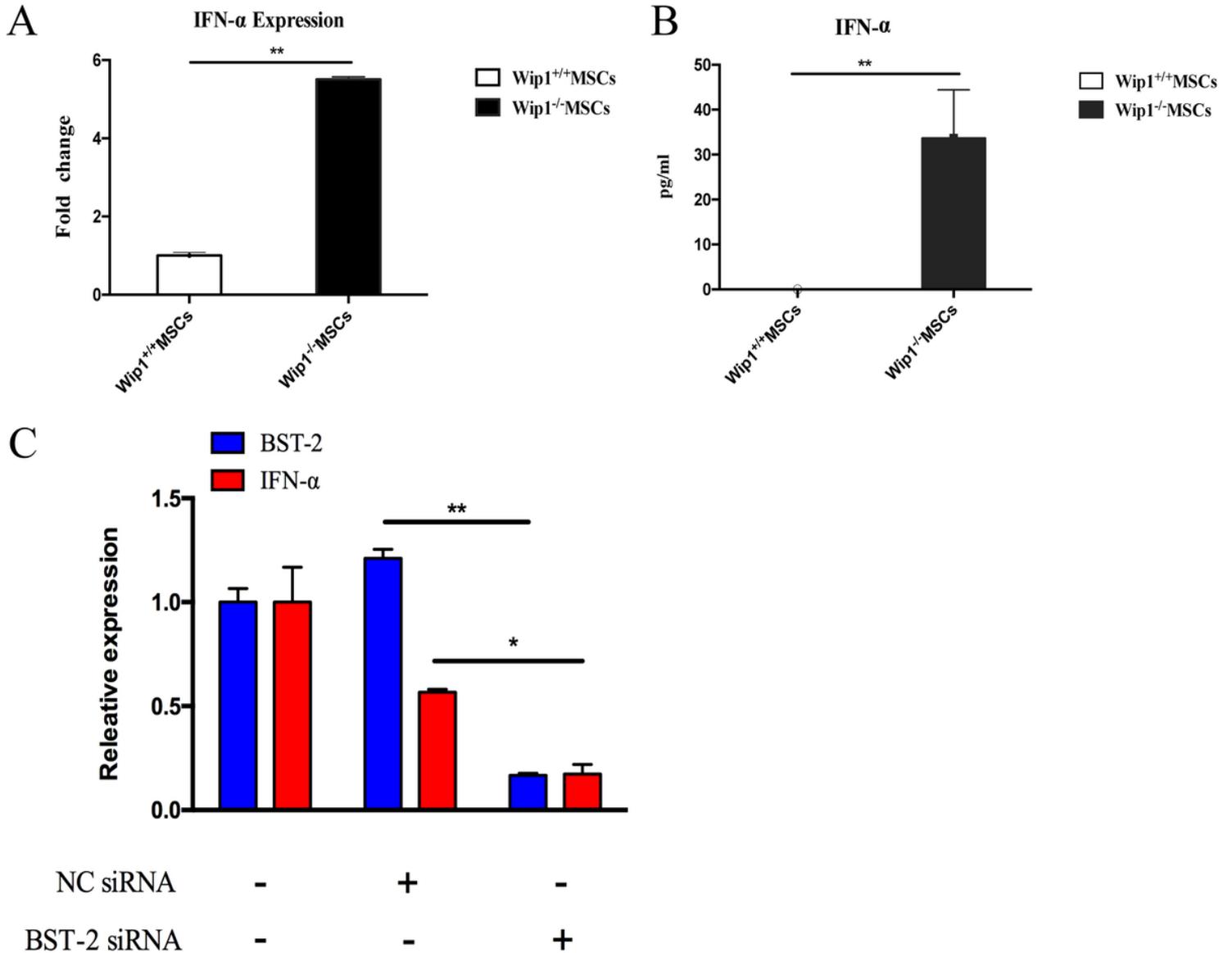


Figure 5

BST2 knockdown inhibited the expression of Wip1 and decreased IFN- α transcription. (A-B) The mRNA and protein levels of IFN- α in Wip1^{+/+} MSCs and Wip1^{-/-} MSCs were detected by qPCR and ELISA. (C) Wip1^{-/-} MSCs were transfected with BST2 siRNA, and the mRNA level of BST2 and IFN- α in Wip1^{-/-}MSCs were measured by qPCR 48 h after transfection. *P < 0.05, **P < 0.01.

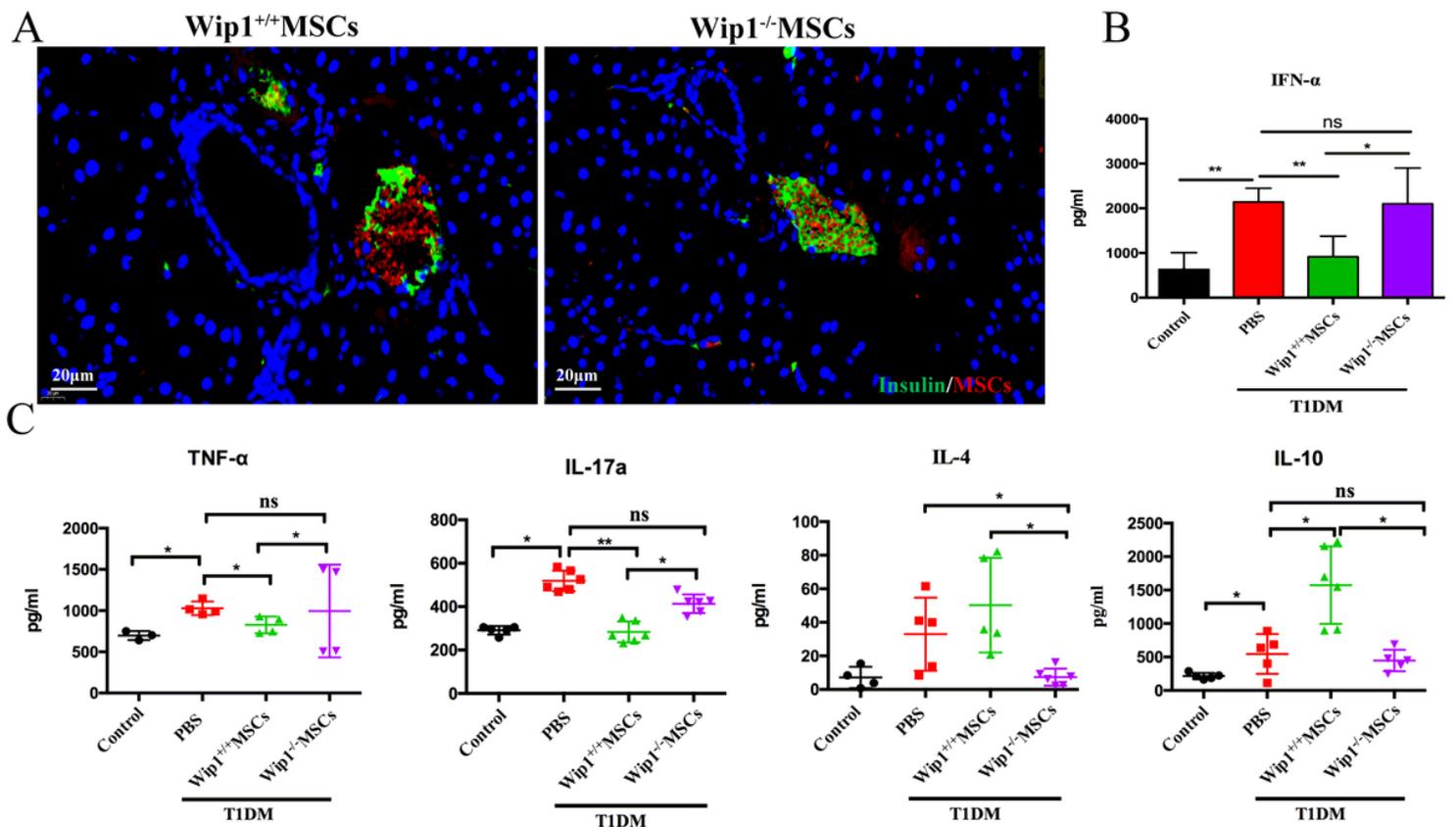


Figure 6

IFN- α secreted by Wip1^{-/-} MSCs enhanced the inflammatory response in the pancreatic microenvironments of T1DM mice. (A) Immunofluorescence staining of pancreatic microenvironment including insulin (green), MSCs (red), and nuclei (blue) showed the size change of insulin and homing of MSC in T1D mice with infusion of Wip1^{+/+} MSCs and Wip1^{-/-} MSCs. (B, C) Expression of IFN- α , TNF- α , IL-17 α , IL-4, and IL-10 in pancreas by ELISA. Scale bar, 20 μ m. *P < 0.05, **P < 0.01.

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